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Docket Number	1435.035PRV	Type a plus sign (+) inside this box >	+
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INVENTOR(s)/APPLICANT(s)

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TITLE OF THE INVENTION (280 characters max)

AMPHIPHILIC SCORPION-LIKE MACROMOLECULES (ASeMs) FOR BIOMEDICAL APPLICATIONS

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ENCLOSED APPLICATION PARTS (check all that apply)

XXX	Specification	Number of Pages	7	XXX	Small Entity Status Claimed
	Drawing(s)	Number of Sheets			Other (specify)

METHOD OF PAYMENT (check one)

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Respectfully submitted,

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TYPED OR PRINTED NAME Robert J. Harris REGISTRATION NO. 37,346

PROVISIONAL APPLICATION FILING ONLY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PROVISIONAL Patent Application of: Kathryn E. Uhrich et al.

Title: AMPHIPHILIC SCORPION-LIKE MACROMOLECULES (ASeMs) FOR BIOMEDICAL APPLICATIONS

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(NEW FILING)

Amphiphilic Scorpion-like Macromolecules (ASeMs) for Biomedical Applications

Background of the Invention

Currently, there is a need for compositions (e.g. compositions comprising nanoscale substrates) and methods that are useful for the sequestration of unoxidized and/or weakly oxidized low-density lipoproteins (LDL). There is also a need for polymeric micellar carriers for drug delivery.

Summary of the Invention

In one embodiment, the invention provides a substrate (e.g. an AScMs) that is useful for the sequestration of unoxidized and/or weakly oxidized low-density lipoproteins (LDL).

In another embodiment the invention provides a method for sequestering LDL *in vitro or in vivo* comprising contacting LDL with a substrate of the invention.

In another embodiment the invention provides a method for sequestering LDL from an animal (e.g. a human) in need of such treatment comprising administering a substrate of the invention to the animal.

In another embodiment the invention provides a method for inhibiting atherosclerotic development in an animal comprising administering a substrate of the invention to the animal.

In another embodiment the invention provides polymeric micellar carriers for drug delivery.

The invention also provides a pharmaceutical composition comprising a substrate of the invention and a pharmaceutically acceptable diluent or carrier.

The invention also provides a substrate of the invention for use in medical therapy.

The invention also provides the use of a substrate of the invention to prepare a medicament useful for sequestering LDL in an animal (e.g. a mammal such as a human).

The invention also provides the use of a substrate of the invention to prepare a medicament useful for inhibiting atherosclerotic development in an animal (e.g. a mammal such as a human).

The invention also provides an AScMs covalently coupled to paclitaxel.

The invention also provides a pharmaceutical composition comprising an AScMs that is covalently coupled to paclitaxel and a pharmaceutically acceptable diluent or carrier.

The invention also provides a method for treating cancer comprising administering an effective therapeutic amount of an AScMs covalently coupled to paclitaxel to an animal (e.g. a mammal or a human).

The invention also provides the use of an AScMs that is covalently coupled to paclitaxel to prepare a medicament that is useful for treating cancer.

The invention also provides synthetic intermediates and procedures described herein that are useful for preparing a substrate of the invention.

Detailed Description

The entire disclosure of the following manuscript (2 pages) entitled "Amphiphilic Scorpion-like Macromolecules (ASeMs) for Biomedical Applications" is included as part of this provisional patent application.

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Amphiphilic Scorpion-like Macromolecules (AScMs) for Biomedical Applications

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ABSTRACT SUMMARY

Amphiphilic scorpion-like macromolecules (AScMs) were designed and synthesized. The two major biomedical applications were explored: polymeric micellar carriers for drug delivery and nanoscale substrates for the sequestration of unoxidized and/or weakly oxidized low-density lipoproteins (LDL). This presentation covers recent development on both aspects.

INTRODUCTION

Amphiphilic macromolecules can form stable micellar aggregates in aqueous solution capable of carrying hydrophobic drugs[1], similar to naturally occurring molecular carriers such as lipoproteins[2]. Polymeric micelles encapsulate the lipophilic drug molecules within the hydrophobic core, solubilizing the drug with the hydrophilic shell in aqueous solution. In addition to compartmentalization of hydrophobic drugs, the amphiphilic macromolecules must meet additional criteria to be effective drug carriers. Biocompatibility, appropriate size (10 nm - 100 nm) for blood circulation, and a low critical micelle concentration (CMC) are important characteristics that lead to improved bioavailability, reduction of toxicity, enhanced permeability across physiological barriers, and substantial changes in drug biodistribution[3]. With a comprehensive understanding of their chemical compositions and corresponding relationship to physical properties, amphiphilic macromolecules can be designed for optimal drug delivery as well as other biomedical applications.

In this work, a series of amphiphilic polymers referred to as amphiphilic scorpion-like macromolecules (AScM) was developed. For biomedical applications, our design criteria for the amphiphilic macromolecules are four-fold. First, a tunable hydrophilic-lipophilic balance (HLB) is desired to match the amphiphilic drug carriers with the drug hydrophobicity to optimize drug-carrier interactions. Second, polymer systems themselves should not cause any undesirable biological complications, such as toxicity and immunogenicity[4]. Third, the polymers should be biodegradable and easily excreted from living systems. Lastly, the inclusion of biological functionality will significantly aid in the selective biomedical applications.

We prepared a series of AScMs that meet these four criteria[5]. **(Figure 1)** The macromolecules are synthesized from mucic acid, monohydroxy-poly(ethylene glycol) (PEG) and acyl chlorides. These AScMs systems are referred to as M_xP_y, in which M denotes mucic acid; x denotes the total carbon number of each acyl chain; P denotes PEG, and y refers to molecular weight of the PEG in thousands. Mucic acid has four hydroxyl groups that are acylated by a series of acyl chlorides with varying chain lengths. The alkyl chain lengths are tuned to tailor the structure and properties of the resulting polymers. The multi-branched hydrophobic domain is proposed to be more efficient in self-assembly in aqueous media than a single hydrophobic block[6,7]. Mucic acid also has two carboxylic acid groups, which can be selectively activated and conjugated to bioactive molecules. Additionally, mucic acid is a naturally occurring compound, which enhances the biocompatibility of the final polymers[8]. Finally, PEG was chosen for its well-known biological significance. With a PEG-based shell, microparticles such as micelles and liposomes as well as nanoparticles can avoid the adsorption of proteins and adhesion of cells in biological media[9].

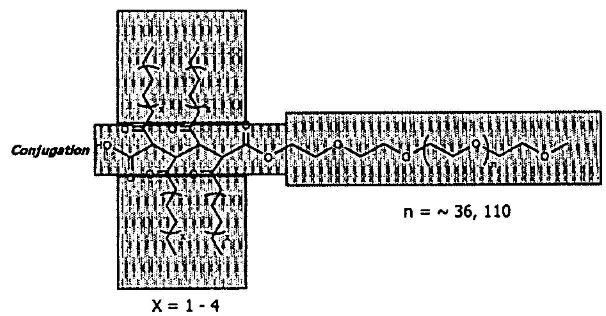


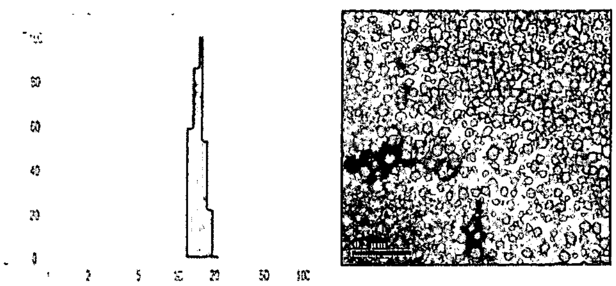
Figure 1. The chemical structure of AScMs

RESULTS AND DISCUSSION

AScMs are synthesized from mucic acid, monohydroxy-poly(ethylene glycol) (PEG) and acyl chlorides. The four hydroxyl groups of mucic acid were acylated using acyl chlorides with varying chain lengths. Zinc chloride was added as catalyst, the reactions were performed using acyl chlorides as solvent at 90 °C. The acylated derivatives of mucic acid were coupled onto the PEG with 1,3-dicyclohexylcarbodiimide (DCC) as coupling agent and 4-(dimethylamino) pyridinium *p*-toluenesulfonate (DPTS) as catalyst to yield the amphiphilic polymers.

Similar to conventional amphiphilic diblock copolymers[10-12], AScMs systems have a hydrophilic block (i.e., PEG) that is modified by a hydrophobic portion (i.e., acylated mucic acid derivatives). Unlike amphiphilic diblock copolymers, the hydrophobic component of the M_xP_y materials is multi-branched. We propose that the extremely hydrophobic, multi-branched domain will contribute to forming a stable aggregation in an aqueous system, which is ultimately a function of HLB. At the concentrations above critical micelle concentration (CMC), AScMs form micelles with the interior hydrophobic core and the hydrophilic chains extending outside. The CMC values for the AScMs polymers range

between 10^{-5} M and 10^{-7} M. The low CMC values illustrate the effectiveness of multi-branched structure for stabilizing micellar aggregates. With such low CMC values, AScMs can form a highly stable micellar aggregates with low rates of dissociation *in vivo*[13]. By dynamic light scattering (DLS) analyses, all micellar aggregates have unimodal size distribution at around 10 ~ 20 nm within ± 2 nm derivations. Additionally, transmission electron microscopy studies (TEM) confirmed such nanoscale particles. (**Figure 2**) The sizes were independent of both PEG and alkyl chain lengths. Above the critical micelle concentrations, the aggregations are quite stable upon dilution. For drug delivery applications, these stable, narrow-distributed nanocarriers are expected to show extravasation efficacy for solid tumor tissues[14] and evade reticuloendothelial system up-take[15].



(a) (b)

Figure 2. (a) Dynamic light scattering studies of AScMs nanocarriers; (b) Transmission electron microscopy of AScMs nanocarriers.

In addition to drug delivery applications, AScMs also show potential as mobile nanoscale substrates for the sequestration of unoxidized and/or weakly oxidized low-density lipoproteins (LDL) for the systematic inhibition of atherosclerotic development[16]. These AScMs as nanocarriers can compete with proteoglycans for LDL binding; to capture LDL before it gets extensively oxidized and transport to cells in its least atherogenic form for controlled cellular uptake and metabolism. Interactions between LDL and the anionic nanocarriers from AScMs were confirmed using both dynamic light scattering and transmission electron microscopy. LDL-AScM complexes of about 60-90 nm in size were detected and visualized supporting the hypothesis that LDL can be sequestered due to electrostatic interactions between the anionic AScMs and the positively charged amino acid residues on the LDL particle[17]. The presence of the AScM hydrophobic core close to the carboxylate functionality may further enhance LDL binding through hydrophobic-hydrophobic interactions. The AScM nanocarriers may encapsulate hydrophobic antioxidants in the core, a topic of future investigation.

Moreover, these AScMs can be functionalized through the carboxylic acid group by coupling with paclitaxel (an antitumor agent) to form a polymer prodrug. This prodrug (as a new AScM) can be further loaded with different lipophilic drug species by physical entrapment, essentially behaves as "double-dose" drug carriers. Attaching the targeting molecules such as folate at the end of hydrophilic PEG block is another step toward targeting drug delivery.

CONCLUSION

A series of amphiphilic scorpion-like macromolecules (AScM) were successfully synthesized based on mucic acid, acyl chlorides and mono-hydroxylated poly(ethylene glycol). We demonstrated that the micellar behavior of these polymeric amphiphiles can be carefully controlled by manipulating the molecular architecture of core-forming hydrophobic components. The multi-branched structure of the hydrophobic region enhances the ability of the macromolecules to self-assemble into highly stable micellar aggregates. The low critical micelle concentrations (as low as 10^{-7} mol/L) and small particle sizes (10 nm to 20 nm) of these AScMs are appropriate, if not ideal, for lipophilic drug delivery and transport. Additionally, AScMs have shown the ability of retaining native and mildly oxidized LDL for the systematic inhibition of atherosclerotic development.

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Substrates of the invention (AScM)s can also be prepared using techniques similar to those described in PCT/US03/17902 (WO 03/103594).

All publications, patents, and patent documents (including PCT/US03/17902) are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

Claims

What is claimed is:

1. A method for sequestering LDL *in vitro* or *in vivo* comprising contacting LDL with an effective sequestering amount of an AScMs.
2. The method of claim 1 wherein the AScMs is an AScMs as shown in Figure 1 hereinabove.
3. The method of claim 1 wherein the AScMs is an AScMs as described in WO 03/103594.
4. The method of claim 1 wherein the AScMs is an AScMs prepared hereinabove.
5. A method for sequestering LDL from an animal comprising administering an effective sequestering amount of an AScMs to the animal.
6. The method of claim 5 wherein the AScMs is an AScMs as shown in Figure 1 hereinabove.
7. The method of claim 5 wherein the AScMs is an AScMs as described in WO 03/103594.
8. The method of claim 5 wherein the AScMs is an AScMs prepared hereinabove.
9. The method of any one of claims 5-8 wherein the animal is a mammal.
10. The method of any one of claims 5-8 wherein the animal is a human.
11. A method for inhibiting atherosclerotic development in an animal comprising administering an effective atherosclerotic development inhibiting amount of an AScMs to the animal.
12. The method of claim 11 wherein the AScMs is an AScMs as shown in Figure 1 hereinabove.
13. The method of claim 11 wherein the AScMs is an AScMs as described in WO 03/103594.
14. The method of claim 11 wherein the AScMs is an AScMs prepared hereinabove.
15. The method of any one of claims 11-14 wherein the animal is a mammal.

16. The method of any one of claims 11-14 wherein the animal is a human.

17. The use of an AScMs to prepare a medicament useful for sequestering LDL in an animal.

18. The use of an AScMs to prepare a medicament useful for inhibiting atherosclerotic development in an animal.

19. The use of claim 17 or 18 wherein the AScMs is an AScMs as shown in Figure 1 hereinabove.

20. The use of claim 17 or 18 wherein the AScMs is an AScMs as described in WO 03/103594.

21. The use of claim 17 or 18 wherein the AScMs is an AScMs prepared hereinabove.

22. The use of any one of claims 17-21wherein the animal is a mammal.

23. The use of any one of claims 17-21 wherein the animal is a human.

24. An AScMs covalently coupled to paclitaxel.

25. The AScMs of claim 24 wherein the AScMs is coupled through a carboxylic acid group of the AScMs.

26. The AScMs of claim 24 or 25 which is loaded with a drug species.

27. The AScMs of claim 26 wherein the drug species is loaded by physical entrapment.

28. The AScMs of any one of claims 24-27 which is attached to a targeting molecule.

29. The AScMs of claim 28 wherein the targeting molecule is folic acid.

30. The AScMs of claim 29 wherein the folic acid is folate attached at the end of a hydrophilic PEG block of th4e AScMs.

31. A pharmaceutical composition comprising an AScMs as described in any one of claims 24-30 and a pharmaceutically acceptable diluent or carrier.

32. A method for treating cancer comprising administering an effective therapeutic amount of an AScMs as described in any one of claims 24-30 to an animal.

33. The use of an AScMs as described in any one of claims 24-30 to prepare a medicament that is useful for treating cancer.